THE SYSTEMATIC RELATIONSHIPS OF ACTINOBACILLUS

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It has been suggested that there is a group relationship between Actinobacillus lignièresi and Pfeifferella mallei. This relationship is based on morphologic, cultural and serologic characters, the evidence for which I shall present. It is pertinent at this time, also, to recall that Stanton and Fletcher pointed out the close relationship existing between Bacillus whitmori and Pfeifferella mallei.

The organism of glanders was first obtained in pure culture by Loeffler and Schütz. The preliminary report of this work was made in 1882 by Struck who was director of the Gesundheitsamt. This report is usually credited to Loeffler and Schütz. In 1886 Loeffler published a detailed account of the work. In both of these publications the common name glanders bacillus (Rotzbacillus) is used to designate the organism. In 1886 Flügge used the name Bacillus mallei. Thus, the species name should be credited to Flügge instead of to Loeffler or to Loeffler and Schütz. In 1916 Buchanan proposed the generic name Pfeifferella, with the single species mallei.

The organism causing actinobacillosis of cattle was described by Lignières and Spitz in 1902, and Brumpt in 1910 proposed the generic name Actinobacillus and the species name lignièresi. In this case also there was a single species within the genus. Whitmore in 1913 described an organism found by him in a “glanders-like” disease of human beings in Rangoon, and suggested the species name pseudomallei. Stanton and Fletcher encountered the same organism and gave the name Bacillus whitmori, stating that the term pseudomallei was invalid without giving a reason.
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for the statement. They also named the disease produced by this organism melioidosis after the name melis which was used by ancient Greek physicians to designate a variety of conditions resembling glanders. According to these workers, Bacillus whitmori is of rodent origin. The similarity between Bacillus whitmori and Pfeifferella mallei has led Topley and Wilson to place Whitmore's organism tentatively in the genus Pfeifferella. However, in the classification of the Society of American Bacteriologists it is put in the genus Flavobacterium. In this classification, therefore, the three organisms in question are put in three separate families.

Although it is my intention primarily to emphasize the relation existing between the Actinobacillus and Pfeifferella mallei, I shall take occasion to confirm the work of Stanton and Fletcher with regard to the cultural characters and serologic relationships of Bacillus whitmori. The fifteen cultures of Actinobacillus used in this study were isolated from infected cattle. Their cultural characteristics and serologic relationships have been described previously (Thompson, 1933). Since there was little variation among them in cultural characters, the three strains for which immune rabbit serums had been prepared were included for the present group study. The strains and sources of the other organisms used are as follows: (1) Bacillus whitmori No. 4799 American Type Culture Collection (No. 1688 National Collection of Type Cultures); (2) Bacillus whitmori No. 4800 American Type Culture Collection (No. 1691 National Collection of Type Cultures); (3) Pfeifferella mallei, Parke-Davis and Company, designated P.D. (represents No. 3 Army Medical School); (4) Pfeifferella mallei, H. K. Mulford Company, designated Mf, and (5) Pfeifferella mallei, Minnesota State Board of Health, designated F.

There is nothing distinctive in the size or staining reactions of the three organisms. All are medium sized Gram-negative rod-shaped organisms. Table 1 gives the cultural characters which were of differential value. The potato medium is the most useful in distinguishing Actinobacillus lignieresi from the other two organisms. On this the growth of Actinobacillus lignieresi is slight or negative, whereas Bacillus whitmori and Pfeifferella mallei
**TABLE 1**

*Differential Cultural Characteristics of Genus Actinobacillus*

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>MOTILITY</th>
<th>GLYCEROL AGAR</th>
<th>GELATINE</th>
<th>BLOOD SERUM</th>
<th>POTATO</th>
<th>INDOL</th>
<th>SUGAR FERMENTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>Smooth white</td>
<td>Not liquefied</td>
<td>Not liquefied</td>
<td>Growth poor</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>Smooth white</td>
<td>Not liquefied</td>
<td>Not liquefied</td>
<td>Growth poor</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>27</td>
<td>-</td>
<td>Smooth white</td>
<td>Not liquefied</td>
<td>Not liquefied</td>
<td>Growth poor</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>99</td>
<td>-</td>
<td>Wrinkled yellow</td>
<td>Rapid liquefaction</td>
<td>Liquefied</td>
<td>Good growth yellow</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>00</td>
<td>-</td>
<td>Wrinkled yellow</td>
<td>Slow liquefaction</td>
<td>Liquefied</td>
<td>Good growth yellow</td>
<td>+</td>
<td>A</td>
</tr>
<tr>
<td>PD</td>
<td>-</td>
<td>Smooth yellow</td>
<td>Not liquefied</td>
<td>Not liquefied</td>
<td>Good growth yellow</td>
<td>+ sl.</td>
<td>0</td>
</tr>
<tr>
<td>Mf</td>
<td>-</td>
<td>Smooth yellow</td>
<td>Slow liquefaction</td>
<td>Not liquefied</td>
<td>Good growth yellow</td>
<td>+ sl.</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>Smooth yellow</td>
<td>Slow liquefaction</td>
<td>Not liquefied</td>
<td>Good growth yellow</td>
<td>+ sl.</td>
<td>A sl.</td>
</tr>
</tbody>
</table>

Cultures 1, 10 and 27 are *Actinobacillus lignieresii*; 99, 00 are *Bacillus whitmori*; Pd, Mf and F are *Pfeifferella mallei*.  

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produce an abundant yellowish growth, which, in the case of *Bacillus whitmori*, was wrinkled. However, smooth strains have been described. The growth of *Actinobacillus lignièresi* on glycerol agar is not pigmented, whereas the other two organisms produce a yellowish growth on this medium.

The sugar fermentation reactions of the three organisms are similar in that no gas is produced. The freshly isolated strains of *Actinobacillus lignièresi* were more active on sugars than the stock strains of *Bacillus whitmori*, although Stanton and Fletcher noted that the latter organism lost some of its fermentative powers after several months on artificial mediums. *Pfeifferella mallei* was the least active on sugars. One of the three strains attacked glucose slightly. Stanton and Fletcher also found that *Pfeifferella mallei* may ferment glucose slowly.

The cultures of *Bacillus whitmori* included in this work were motile in young broth cultures and liquefied gelatine in three to four days at 37°C., thus differing from the cultures of *Actinobacillus lignièresi* and *Pfeifferella mallei*. It was found also that *Bacillus whitmori* liquefied blood serum rapidly at 37°C. This does not agree with the statement by Topley and Wilson that *Bacillus whitmori* fails to liquefy blood serum. The cultures of *Bacillus whitmori* agreed in all other respects with the published descriptions of this organism, and, as will be seen, were serologically related to *Pfeifferella mallei*.

The serologic relationship of the three organisms was tested by means of cross-agglutination tests, using immune rabbit serums. In addition, the serums of immune rabbits and of one human being infected with Actinobacillus, were tested by the method of fixation of complement, using as antigen a preparation of mallein obtained from one of the manufacturers of biological products. Table 2 shows the results of cross agglutination tests. It can be seen that serums prepared with cultures of Actinobacillus often contain agglutinins for *Bacillus whitmori* and *Pfeifferella mallei*, and conversely that serums prepared with cultures of *Bacillus whitmori* and *Pfeifferella mallei* often contain agglutinins for *Actinobacillus lignièresi*. The two strains of *Bacillus whitmori*, and the strains P.D. and F of *Pfeifferella mallei* are similar. On
the other hand the strain Mf of *Pfeifferella mallei* seems to be more like strain 10 of *Actinobacillus*. These serums were also tested with strains of *Alcaligenes abortus*, *Pasteurella tularensis* and *Klebsiella rhinoscleromatis*, to find out if there might be any evidence of group reactions. Results were negative in all cases.

The complement-fixation test does not distinguish among the three organisms as is shown by the following tests. Using a preparation of mallein as antigen, the following serums gave a strongly positive reaction: (1) human serum from a case of infection with *Actinobacillus*; (2) serums of three rabbits immunized with cultures of *Actinobacillus*; (3) serums of two rabbits immunized with cultures of *Pfeifferella mallei*, and (4) serum of one rabbit immunized with a culture of *Bacillus whitmori*.

Besides the cultural and serologic characters of the three organisms, which indicate that they are quite closely related, they are similar in other ways. Each organism is the cause of a disease of animals which may be transmitted to man. Each organism is said to produce the Strauss reaction in male guinea pigs. There is also a general similarity in the manner in which each of the three organisms localizes in its animal host. Lymphatic and pulmonary involvement is common. In acute cases of glanders and melioidosis there appears to be a dissemination by the hematogenous route with the formation of multiple necrotic foci,

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>NAME</th>
<th>RABBIT SERUMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td><em>Actinobacillus lignieresii</em></td>
<td>5,120</td>
</tr>
<tr>
<td>10</td>
<td><em>Actinobacillus lignieresii</em></td>
<td>320</td>
</tr>
<tr>
<td>27</td>
<td><em>Actinobacillus lignieresii</em></td>
<td>1,280</td>
</tr>
<tr>
<td>99</td>
<td><em>Bacillus whitmori</em></td>
<td>20</td>
</tr>
<tr>
<td>00</td>
<td><em>Bacillus whitmori</em></td>
<td>40</td>
</tr>
<tr>
<td>PD</td>
<td><em>Pfeifferella mallei</em></td>
<td>40</td>
</tr>
<tr>
<td>Mf</td>
<td><em>Pfeifferella mallei</em></td>
<td>40</td>
</tr>
<tr>
<td>F</td>
<td><em>Pfeifferella mallei</em></td>
<td>0</td>
</tr>
</tbody>
</table>
whereas in actinobacillosis the disease runs a chronic course in cattle which may be compared to the chronic type of glanders in horses known as farcy. In both cases the lymphatic structures are involved primarily, and the glands tend to break down and suppurate.

In view of the striking similarity which is exhibited by the three organisms now known in the classification of the Society of American Bacteriologists as Actinobacillus lignieresii, Pfeifferella mallei and Flavobacterium pseudomallei, it is recommended that the two last named organisms be placed in the genus Actinobacillus Brumpt, since this name has priority over the name Pfeifferella proposed by Buchanan. It is perhaps unfortunate that Actinobacillus has been used for this group which has so little in common with the Actinomycetaceae. It is further recommended that the proposed genus Actinobacillus be placed in the family Mycobacteriaceae in the position at present occupied by Pfeifferella. The following definition for the genus is suggested and a key is offered for the separation of species:

**GENUS V ACTINOBACILLUS BRUMPT, 1910**

Medium sized aerobic Gram-negative rods, staining poorly, sometimes forming threads and showing a tendency toward branching. Good growth on ordinary culture medium. Produce acid but no gas in carbohydrates. Cause diseases of animals which may be transmitted to man. The type species is *Actinobacillus lignieresii* (Lignieres and Spitz, 1902, Brumpt 1910).

**Key to the species of genus Actinobacillus**

1. Nonmotile
   a. Growth slight or negative on potato.
      aa. Good growth on potato.

2. Motile
   a. Good growth on potato.

   1. *Actinobacillus lignieresii*
   2. *Actinobacillus mallei*
   3. *Actinobacillus pseudomallei*

**SUMMARY**

A study has been made of three strains of *Actinobacillus lignieresii* isolated from cattle in the United States, two strains of
Actinobacillus pseudomallei received from abroad, and three strains of Actinobacillus mallei obtained from laboratories in this country.

The results of this study confirm the report of Stanton and Fletcher concerning the close relationship existing between Actinobacillus mallei and Actinobacillus pseudomallei. It is also shown that Actinobacillus lignieresii is related to the above named organisms morphologically, culturally and serologically.

The generic name Actinobacillus has priority for this group of organisms, and is recommended. It is also recommended that the genus Actinobacillus be removed from the family Actinomycetaceae and be placed in the family Mycobacteriaceae in place of genus Pfeifferella which it supplants.

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Whitmore, A.: Quoted by Stanton and Fletcher.